

# Mitochondrial antibody test—a clue to diagnosis of primary biliary cirrhosis

## REPORT OF TWO INTERESTING CASES

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**P**RI-MARY biliary cirrhosis, described by Hanot<sup>1</sup> in 1875 as a chronic intrahepatic cholestasis occurring mainly in women, was largely neglected as an entity until the publication of the complete clinical description by MacMahon,<sup>2</sup> Ahrens and associates,<sup>3</sup> Sherlock,<sup>4</sup> and others. The studies in pathology by Albot, Nezelof, and Lunel,<sup>5</sup> Caroli and others<sup>6</sup> in France, and by Rubin, Schaffner, and Popper<sup>7</sup> in this country first with the light microscope and then with the electron microscope, described the lesion to be at the level of the small intrahepatic ducts and ductules. None of the studies defined the pathogenesis of this peculiar and fatal syndrome.

Since the diagnosis of primary biliary cirrhosis requires the absence of obstruction of the extrahepatic and large intrahepatic biliary ducts, and requires histologic confirmation of intrahepatic cholestasis, in questionable cases the examining clinician is often forced into advising an exploratory laparotomy. In order to avoid surgery, new means of diagnosis have been sought. Certain clinical and pathologic features of primary biliary cirrhosis have led many investigators to focus on the immunologic aspects of this puzzling hepatic disease. Paronetto, Schaffner, and Popper<sup>8</sup> found accumulation of IgM macroglobulins in the mesenchymal cells in the portal spaces and fibrous septa as well as antiductular antibodies in patients who had primary biliary cirrhosis. Albano and associates<sup>9</sup> and Bevan and associates<sup>10, 11</sup> pointed out the high concentrations of serum IgM found in primary biliary cirrhotic patients. The latex agglutination test for rheumatoid factor, the antinuclear test, the complement-fixation test with nuclei, and smooth-muscle antibody tests were found to be positive in many of the patients. However, the lack of specificity of such tests makes them unreliable

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for the diagnosis of this disease, since many of these immunologic tests are positive also in patients who have various other hepatic diseases.<sup>11-16</sup>

This report presents the results of an immunologic test introduced in 1965 by Walker and associates<sup>17</sup>: the immunofluorescent mitochondrial antibody test (M-test). The mitochondrial antibody was first detected by these investigators purely in an empiric fashion and, as yet, no satisfactory explanation has been offered for its presence in patients with primary biliary cirrhosis. Our interest in it stems from the hope that it might add a significant element to the diagnosis of primary biliary cirrhosis.

### Material and methods for studying mitochondrial antibodies

Blocks of fresh rat kidney were quickly frozen with liquid nitrogen, and the frozen tissue was sectioned (4 to 6  $\mu$  thick) on a cryostat. The sections were treated first with patient's serum and later with fluorescent antihuman  $\gamma$ -globulin (from Hyland Laboratories, Los Angeles, California). Then, under the fluorescent microscope, the sections were examined for the characteristic apple-green fluorescence. In a positive test, the cytoplasm of the tubular cells had a typically speckled fluorescence.

The M-test was performed on sera of 55 patients. Nine of the patients had primary biliary cirrhosis, while 46 had various other abnormal conditions of the liver (*Table 1*).

The diagnosis of primary biliary cirrhosis was made in accordance with the following three widely accepted criteria: (1) presence of clinical and laboratory features of chronic cholestasis, (2) evidence of patent extra-hepatic and large intrahepatic biliary ducts on operative cholangiograms, and (3) histopathologic findings in the liver compatible with the diagnosis of primary biliary cirrhosis. The results of the immunologic test—which was performed without prior knowledge of the clinical diagnosis—did not change our previous diagnosis for any of the patients.

### Results

The pertinent findings in the nine patients with primary biliary cirrhosis are listed in *Tables 2 and 3*.

Of the nine patients with primary biliary cirrhosis, seven had positive M-tests and two had negative results. One patient, reported as having positive results in 1967, recently had negative results. The serum of 1967 was retested and gave negative results, although the possibility cannot be excluded that long-term storage caused inactivation of the serum. We have included this questionable case as one of the two false-negative tests. The M-test was repeated three or more times at various intervals in most patients, before and after steroid or immunosuppressive therapy; neither the quality of the tests nor the titers changed in the patients during those periods. It is interesting that all patients with primary biliary cirrhosis and positive tests had titers of 1:160 or higher (*Table 1*). The other patient with a nega-

**Table 1.**—*Results of the mitochondrial antibody test (M-test) in 55 patients*

Primary disease	Patients, number		
	Tested	With positive results	Titer
Primary biliary cirrhosis	9	7	1:160
Control subjects, total number	46	3	
Extrahepatic obstruction	10	1	1:40
Active chronic hepatitis (lupoid hepatitis)	8	1	1:80
Viral hepatitis	4	0	
Cholestatic drug-induced jaundice	2	0	
Sclerosing cholangitis (in patients with ulcerative colitis or Crohn's disease)	2	0	
Other	20	1	1:40
Total	55	10	

**Table 2.**—*Clinical findings in nine patients with primary biliary cirrhosis*

Patient			Clinical findings* in biliary cirrhosis				
No.	Sex	Age, yr	Jaundice	Pain	Pruritus	Hepato-megaly	Xanthoma
1	F	38	++	—	+	+++	++
2	F	57	+	±	—	+++	+
3	M	44	±	—	++	+	—
4	F	48	+	±	±	+	—
5	F	61	—	—	++	++	++
6	F	50	+	—	++	++	++
7	M	50	+	—	+	+	+
8	F	46	+	—	+	+++	+++
9	F	60	+	—	+	++	+

\* Graded from + to +++; — is negative; ± is questionable.

tive test had the typical clinical features of primary biliary cirrhosis, but the test remained negative over a three-year period. During that time the IgM immunoglobulin concentration increased, and also a positive smooth-muscle antibody (titer 1:1000), suggestive of some immunologic reaction of the type seen usually in primary biliary cirrhosis.

There were three false-positive tests. One patient with a titer of 1:80 had lupoid hepatitis (chronic active hepatitis). One patient, with a titer of 1:40, had secondary biliary cirrhosis due to recurrent stones in the common bile duct. One patient, with a false-positive test (titer of 1:40), had intrahepatic granulomatous disease of unknown cause. Because his clinical picture was suggestive of cholestasis, with high alkaline phosphatase and serum cholest-

**Table 3.**—*Pathologic and roentgen findings in nine patients with primary biliary cirrhosis*

Patient no.	Serum values			Liver biopsy diagnosis	Percutaneous cholangiogram	Operation	Operative cholangiogram
	Bilirubin, mg/100 ml	Alkaline phosphatase, King-Armstrong units	Cholesterol, mg/100 ml				
1	11.3	79	1300	Active biliary cirrhosis	None	Yes	Normal
2	10.4	160	900	Cholangiolitic hepatitis	Not successful	Yes	Normal
3	1.4	14	492	Portal inflammation and fibrosis without bile stasis	None	None	None
4	3.0	37	290	Biliary cirrhosis with cholangiolitis	Not successful	Yes	Normal
5	2.1	150	345	Portal inflammation and mild periportal inflammation, no bile stasis	Not successful	None	None
6	3.0	107	1010	Cirrhosis, portal, distributional, biliary type	None	None	None
7	2.6	169	1275	Cholangiolitic hepatitis	Not successful	Yes	Normal
8	6.0	214	925	Chronic pericholangiolitis, portal infiltrate, mainly lymphocytic	Not successful	Yes	Normal
9	5.0	112	625	Biliary cirrhosis with cholangiolitis	None	Yes	Normal

terol concentrations, he underwent operation. The gallbladder was normal; the operative cholangiogram revealed normal and patent bile ducts; and the surgical biopsy of the liver revealed the noncaseating granulomatous lesions.

Among the 10 patients with extrahepatic obstruction (six of them with secondary biliary cirrhosis) only one had a positive M-test, the same patient already mentioned.

The M-test was negative in the other patients, including seven with lupoid hepatitis, two with sclerosing cholangitis (associated with Crohn's disease of the colon in one patient, and chronic ulcerative colitis in the other), four patients with viral hepatitis (after a blood transfusion in one of them), two patients with chronic cholestasis due to chlorpromazine hydrochloride therapy, two with postnecrotic cirrhosis, two with collagen disease, two with nutritional cirrhosis, one patient with sarcoidosis of the liver, and 12 other control patients.

Two interesting cases of patients who had positive M-tests are summarized: one patient had a diagnosis of primary biliary cirrhosis, and the other a diagnosis of secondary biliary cirrhosis.

## Report of cases

**Case 1.** A 38-year-old, Caucasian, American woman lived in Libya, Africa, for two and one-half years. She was well until December 1965 when anorexia and painless jaundice developed, without fever. The diagnosis was infectious hepatitis, inasmuch as 10 persons in the American community were ill with jaundice. The other nine patients recovered, but jaundice persisted in the patient we report here and, consequently, she returned to the United States and was referred to the Cleveland Clinic for further study. At that time, the urine was dark; she had acholic stools and increasing pruritus, decrease in appetite, with a weight loss of 20 lb. in five months; and was notably weak. In 1958, she had undergone cholecystectomy; there were stones in the gallbladder and some "gravel" in the common bile duct. There was no drug intake before the onset of jaundice. On physical examination on May 19, 1966, she was icteric, the liver was huge and palpable in the pelvic area, firm, smooth, and not tender. There were xanthomas on the hands, eyelids, and the soles of the feet. Laboratory data are summarized in *Table 4*. The immunoglobulins were increased, mainly the IgM: IgG was 1600 mg per 100 ml (normal, 500 to 1500); IgA was 640 (normal 50 to 150); IgM was more than 1000 mg per 100 ml (normal 50 to 100). The L.E. cell test was negative, as was the antinuclear factor (ANF) test. The HIM (hepatitis-infectious mononucleosis) test was positive at a dilution of 1:10. The rectal biopsy specimen was normal.

In view of her history of common bile duct stones and of laboratory findings suggestive of cholestasis or obstruction, laparotomy was performed. At the operation, the common bile duct was patent, and an operative cholangiogram was normal. A liver biopsy specimen taken at the time of operation showed active biliary cirrhosis. The patient was discharged from the hospital with the advice to take prednisone, 20 mg a day, choline, vitamins, and a high-protein and high-carbohydrate diet. She did well, but stopped the regimen in December 1966. She stayed well until May 1967, when again she noted jaundice, light stools, and dark urine. Examination and laboratory test results were unchanged (*Table 4*). The fluorescent test for smooth-muscle antibody was weakly positive at a dilution of 1:10, but the M-test was strongly positive at a dilution of more than 1:160. The serum immunoglobulin content was high, with the IgM (930 mg per 100 ml). The patient was given 100 mg of azathioprine\* daily plus 20 mg of prednisone.

\* *Imuran (azathioprine)* kindly supplied by the Burroughs Wellcome & Co. (U.S.A.) Inc., 1 Scarsdale Road, Tuckahoe, New York 10707.

**Table 4.**—*Case 1—laboratory results*

Date	Serum bilirubin, mg/100 ml	Serum alkaline phosphatase, King-Armstrong units	SGOT, units	Serum albumin, mg/100 ml	Serum globulin, mg/100 ml	Prothrombin time, sec	Mitochondrial antibody, titer	Serum cholesterol, mg/100 ml	Immunoglobulin M fraction, mg/100 ml
January 1966	6.2	55	300	—	—	12/12	—	—	—
May 1966	11.3	79	200	3.1	3.1	12/12	—	1300	1000
May 1967 (no steroid since Dec. 1966)	6.6	106	250	2.7	2.6	12/12	1:160	900	930
October 1967 (steroid and azathioprine therapy)	1.4	35	152	2.9	5.0	12/12	—	450	1055
August 1968 (steroid and azathioprine therapy)	2.4	37	185	3.4	2.7	12/12	1:160	—	520

The patient returned for progress studies in October 1967 and again in August 1968 while adhering to the prescribed regimen. She felt progressively better, and when seen in August 1968 was entirely asymptomatic. Results of the physical examination remained unchanged.

The laboratory studies showed a decrease in serum bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase (SGOT), cholesterol, and IgM values (*Table 4*). The ANF test was positive in a dilution of 1:80 in October 1967, and in a dilution of 1:1 in August 1968. A needle biopsy of the liver performed on both occasions showed no change from the wedge biopsy specimens obtained at operation in May 1966. The patient was maintained on the same treatment, including 100 mg of azathioprine and 20 mg of prednisone daily.

If, in 1966, we had been aware of the value of this mitochondrial test, the findings of a strongly positive M-test and a high IgM value, in addition to the epidemiologic context of hepatitis, would have strengthened our clinical impression of intrahepatic cholestasis so that surgical exploration might have been avoided.

**Case 2.** A 76-year-old Caucasian woman was first examined at the Cleveland Clinic in November 1967 because of a three-year history of anorexia, pruritus, jaundice, and a 35-lb. loss in weight. Her history was negative, with no hepatitis, jaundice, or alcohol intake in the past, and no medication before the onset of jaundice. Icterus apparently occurred insidiously without pain, fever, or chills.

In 1964 she underwent operation, at which time an enlarged liver with areas of fibrosis, a dilated common bile duct, and a thick-walled gallbladder containing many stones were found; several biliary calculi were removed from the common bile duct, and the ampulla of Vater was dilated. Resistance of the ampulla of Vater was encountered and, because of scarring, a sphincterotomy was performed. The operative cholangiogram at the time showed good emptying of the common bile duct, and a T-tube was inserted. After the operation, she felt better, gained some weight, but pruritus and jaundice continued with intermittent anorexia, without pain, fever, or chills.

In November 1967 at the physical examination she was jaundiced; the liver was four

fingerbreadths under the right costal margin, firm but not tender. The spleen was three fingerbreadths below the left costal margin. Laboratory tests revealed: serum cholesterol, 310 mg per 100 ml; alkaline phosphatase, 46 King-Armstrong units; SGOT, 162 mg per 100 ml; serum bilirubin, 3.7 mg per 100 ml; blood hemoglobin, 13.8 g per 100 ml; leukocyte count, 7800 per cubic millimeter; prothrombin time, 13 sec (control was 12 sec). The smooth-muscle antibody test was positive at 1:10 serum dilution, and the M-test was positive at 1:20; the M-test a few months later, when repeated, was still positive at a dilution of 1:40. The barium roentgenographic studies of the esophagus, and esophagoscopy showed extensive varices. The liver biopsy specimen showed finely nodular cirrhosis.

This is a typical case of secondary biliary cirrhosis due to extrahepatic obstruction (stones), with a false-positive M-test.

## Discussion

In 1965, Walker and associates<sup>17</sup> reported their results of the immunofluorescent M-test, as a new immunologic method, in 32 patients with primary biliary cirrhosis, and in 21 patients with proved obstruction of the main bile ducts. The M-test provided a clear-cut distinction between the two entities, since it was positive in all patients with primary biliary cirrhosis and negative in all patients with obstruction. Doniach and associates<sup>18, 19</sup> subsequently found the test was not so specific, and in one of their recent studies of 41 patients with primary biliary cirrhosis and 800 control patients, the M-test was positive in 98 percent of the former, but positive results also were obtained in a few cases of extrahepatic obstruction and other abnormal conditions.

Kantor and Klatskin<sup>20</sup> performed the M-test on 30 patients with primary biliary cirrhosis, and on 377 control patients, including 105 with biliary obstruction. The test was positive in 83.3 per cent of those who had primary biliary cirrhosis.

Our series of nine patients is too small for statistical analysis, but the fact that the M-test was positive in only seven of the nine patients confirms the previous finding that it is not positive in all cases of primary biliary cirrhosis.

The discrepancies between the results in different series could be due to differences in technic. Doniach and associates<sup>18</sup> used human kidney as tissue substrate; Paronetto, Schaffner, and Popper<sup>12</sup> used human thyroid, monkey gastric mucosa and submaxillary glands; and Kantor and Klatskin<sup>20</sup> used rat kidney. Other differences include the serum dilutions used and the types of fluoresceinated antibody used in the different studies.

The mitochondrial antibody is neither tissue nor species specific; the antibody is directed against the mitochondrion, and any tissue containing enough mitochondria will react positively. The proximal and distal tubules of the kidneys provide an excellent and rich source of mitochondria.

The lack of absolute specificity of the M-test is illustrated by the false-positive results obtained in a few control patients whether or not they had hepatic disease. Kantor and Klatskin<sup>20</sup> found false-positive results in 1.3

percent of the control patients (all of whom had hepatic disease), and no positive test among the group of 105 patients with extrahepatic obstruction, or among the patients with collagen disease or hepatitis. Doniach and her group<sup>18</sup> found two positive tests in the 28 patients with extrahepatic obstruction studied in 1966; whereas, in 1965 they<sup>17</sup> had reported the test to be negative in all 21 patients with extrahepatic obstruction. In 1966, they also found a higher incidence of positive tests in the control group: 28 percent of patients with chronic active hepatitis, 31 percent of those with cryptogenic cirrhosis, about 20 percent of those with collagen disease, 2 percent of (random) hospital patients, and 1 percent of patients with infectious hepatitis. Our results seem to correlate better with the later findings of Doniach and associates<sup>18</sup> than their earlier results.<sup>17</sup> However, it should be stressed that the titers in the seven positive M-tests in patients with primary biliary cirrhosis were all 1:160 or greater. The false-positive M-tests in patients without primary biliary cirrhosis were positive in lower dilutions: 1:80, 1:40 and 1:40.

Our study, as well as the studies of Doniach and her group,<sup>18</sup> does not clarify the pathogenesis of primary biliary cirrhosis, or the reactivity of the serum to the M-test. The high incidence (40 percent) of a drug intake among Kantor and Klatskin's<sup>20</sup> series of patients with primary biliary cirrhosis does not correlate with our study in which this relationship could be implicated in only one patient with primary biliary cirrhosis and a positive M-test.\* In our series, two control patients with chronic jaundice induced by chlorpromazine had cholestasis leading to biliary cirrhosis, and neither had a positive M-test. None of the seven patients with drug-induced jaundice studied by Doniach and associates<sup>18</sup> had a positive M-test, which is in contrast with the positive M-test reported by Kantor and Klatskin<sup>20</sup> of a patient who had biliary cirrhosis presumably induced by chlorpromazine. A high percentage of positive M-tests has been reported for the sera from patients with halothane-induced hepatitis.<sup>21</sup> We have had no experience with halothane-induced hepatitis. These observations speak against the hypothesis of drugs being a major factor in the pathogenesis of primary biliary cirrhosis as suggested by Kantor and Klatskin.<sup>20</sup> The cause of primary biliary cirrhosis is still not known. Rarely, hepatotoxic drugs, viral hepatitis, or chronic ulcerative colitis may appear to be etiologic in individual patients.

The case of patient 1 of our report, who is strongly suspected of having a history of viral hepatitis, suggests a relationship between viral hepatitis and primary biliary cirrhosis, and may explain the difficulties encountered sometimes in the diagnosis as suggested by Datta, Sherlock, and Scheuer<sup>22</sup> and by Jones and Tisdale.<sup>23</sup> The various positive immunologic tests in patients with primary biliary cirrhosis, suggest only an alteration or activa-

\* This patient had been taking Sleep-eze, a proprietary drug containing 25 mg of methylpyridine and 0.125 mg of hyosine hydrobromide.



tion of the immune mechanisms, with peculiar reactivity to possibly different etiologic factors.

The positive M-test is not pathognomonic of primary biliary cirrhosis. Its real value is that when it is evaluated with other typical clinical and laboratory features of primary biliary cirrhosis, the M-test may make it possible to avoid surgical exploration. The fact that the significance of the immunologic mechanism involved in this test is not fully understood should not preclude its pragmatic use.

## Summary

Fifty-five patients were studied by the immunofluorescent mitochondrial antibody test (M-test). Of nine patients with primary biliary cirrhosis, seven had positive tests with titers higher than 1:60, while two had negative tests. Of 10 patients with extrahepatic obstruction (six of them with secondary biliary cirrhosis), one had a positive M-test with a titer of 1:40, in a typical case of secondary biliary cirrhosis. Two interesting case reports are presented. A positive M-test, with a high titer, might change our clinical impression to a more confident diagnosis, and thus preclude surgical exploration of the biliary ducts. The nature of primary biliary cirrhosis seems to be more that of a syndrome, with a characteristic histopathologic and clinical picture and unique laboratory features, than that of a simple disease of specific etiology.

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